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L5	l2 same l4	1	L5
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NEWS 4	Feb 01	DKILIT now produced by FIZ Karlsruhe and has a new update frequency
NEWS 5	Feb 19	Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6	Mar 08	Gene Names now available in BIOSIS
NEWS 7	Mar 22	TOXLIT no longer available
NEWS 8	Mar 22	TRCTHERMO no longer available
NEWS 9	Mar 28	US Provisional Priorities searched with P in CA/CAplus and USPATFULL
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NEWS 18	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 19	Jun 03	New e-mail delivery for search results now available
NEWS 20	Jun 10	MEDLINE Reload
NEWS 21	Jun 10	PCTFULL has been reloaded
NEWS 22	Jul 02	FOREGE no longer contains STANDARDS file segment

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=> s mastitis?
L1         16061 MASTITIS?

=> s phytase?
L2          3454 PHYTASE?

=> s l1 and l2
L3             1 L1 AND L2

=> d ab,bib

L3   ANSWER 1 OF 1  CA  COPYRIGHT 2002 ACS
AB   A novel method for preventing/remedying mammalian mastitis and a
     preventive/remedial compn. for mastitis, aiming at preventing
     milk yield lowering caused by the onset of mammalian mastitis.
     The method comprises administering phytase to mammals.
AN   131:54021  CA
TI   Method for preventing/remedying mastitis
IN   Hokase, Masanobu
PA   Kyowa Hakko Kogyo Co., Ltd., Japan
SO   PCT Int. Appl., 20 pp.
     CODEN: PIXXD2
DT   Patent
LA   Japanese
FAN.CNT 1
     PATENT NO.         KIND  DATE           APPLICATION NO.  DATE
     ---------------    ----  --------       ---------------  --------
PI   WO 9932144          A1   19990701       WO 1998-JP5749   19981218
         W:  AU, CA, JP, NZ, US
         RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE
     CA 2314865          AA   19990701       CA 1998-2314865  19981218
     AU 9916842          A1   19990712       AU 1999-16842    19981218
     AU 747111           B2   20020509
     EP 1043028          A1   20001011       EP 1998-961423   19981218
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, FI
PRAI JP 1997-351220     A    19971219
     WO 1998-JP5749     W    19981218
RE.CNT  9     THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8: Entry 31 of 39 File: USPT May 11, 1999

DOCUMENT-IDENTIFIER: US 5902581 A
TITLE: Xylanase from acidothermus cellulolyticus

Detailed Description Paragraph Right (3):
The cereal-based feed according to the present invention may be provided to animals such as turkeys, geese, ducks, sheep and cows. It is however particularly preferred that the feed is provided to pigs or to poultry, and in particular broiler chickens. The cereal-based feed preferably includes 0.00001-10 g of xylanase protein per kilo of the feed; more preferably includes about 0.0001-1 g of xylanase protein per kilo of the feed; and most preferably 0.001-0.1 g of xylanase protein per kilo of the feed. The cereal-based feed comprises at least 20% by weight of cereal. More preferably, it should include at least 30% by weight of the cereal, and most preferably at least 50% by weight of the cereal. The cereal can be any of those previously mentioned, with wheat being particularly preferred.

Detailed Description Paragraph Right (6):
The feed provided by the present invention may also include other enzyme supplements such as one or more of .beta.-glucanase, glucoamylase, mannanase, .alpha.-galactosidase, phytase, lipase, .alpha.-arabinofuranosidase, protease, .alpha.-amylase, esterase, oxidase, oxido-reductase and pectinase. It is particularly preferred to include a protease as a further enzyme supplement such as a subtilisin derived from the genus Bacillus. Such subtilisins are for example described in detail in U.S. Pat. No. 4,760,025.

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L8: Entry 20 of 39 File: USPT Oct 17, 2000

DOCUMENT-IDENTIFIER: US 6132716 A
TITLE: Thermostable xylanases from Microtetraspora flexuosa as a feed additive

Detailed Description Paragraph Right (35):
The cereal-based feed according to the present invention may be provided to animals such as turkeys, geese, ducks, sheep and cows. It is however particularly preferred that the feed is provided to pigs or to poultry, and in particular broiler chickens.

Detailed Description Paragraph Right (40):
The feed provided by the present invention may also include other enzyme supplements such as one or more of .beta.-glucanase, glucoamylase, mannanase, .alpha.-galactosidase, phytase, lipase, .alpha.-arabinofuranosidase, protease, .alpha.-amylase and pectinase. It is particularly preferred to include a protease as a further enzyme supplement such as a subtilisin derived from the genus Bacillus. Such subtilisins are for example described in detail in U.S. Pat. No. 4,760,025.

L8: Entry 27 of 39 File: USPT Jan 25, 2000

DOCUMENT-IDENTIFIER: US 6017530 A
TITLE: Phospholipases in animal feed

Brief Summary Paragraph Right (13):
In recent years, the feed industry has started to use industrially produced enzymes to complement enzymes produced in the gastrointestinal tract of the animals. Examples comprise phytases, .alpha.-amylases, proteases, and various plant cell wall degrading enzymes. However, nowhere in the prior art has the direct addition of phospholipases to animal feed for the purpose of promoting the growth of animals been described since the animals themselves already secrete large amounts of these enzymes in the upper part of the small intestine.

Brief Summary Paragraph Right (33):
Apart from monogastric animals, phospholipase A2 may also be used advantageously in polygastrics. During early lactation of high producing dairy cows for example, it is of interest to include high levels of fat in their diets to compensate in part for the large negative energy balance. From literature it is known that the digestibility of fatty acids in the GI-tract of dairy cows varies as a function of inter alia ration composition and source of fat. Digestibilities of fatty acids have been found to vary between 87% for a diet containing 500 g of saturated fat high in palmitic acid (C16:0) to 64% for a diet containing 1000 g of saturated fat high in stearic acid (C18:0) (Weisbjerg et al., Acta Agric. Scand. Section A, Animal Sciences 42 p. 115-120, 1992).

Brief Summary Paragraph Right (34):
A large part of the variation in digestibility of fatty acids in dairy cows is explained by variations in digestibility in the small intestine (Ibid, p.114-120). Action of phospholipase A2 in the small intestine of dairy cows is likely to enhance the digestibility of fatty acids.

Other Reference Publication (11):
Weisbjert et al., "Digestibility of Fatty Acids in the Gastroinestinal Tract of Diary Cows Fed with Tallow of Saturated Fats Rich in Stearic Acid or Palmitic Acid" Acta Agric. Scand. Section A, Animal Sciences 42:114-120 (1992).

L8: Entry 18 of 39 File: USPT Feb 6, 2001

DOCUMENT-IDENTIFIER: US 6183739 B1
TITLE: Phospholipases in animal feed

Brief Summary Paragraph Right (13):
In recent years, the feed industry has started to use industrially produced enzymes to complement enzymes produced in the gastrointestinal tract of the animals. Examples comprise phytases, .alpha.-amylases, proteases, and various plant cell wall degrading enzymes. However, nowhere in the prior art has the direct addition of phospholipases to animal feed for the purpose of promoting the growth of animals been described since the animals themselves already secrete large amounts of these enzymes in the upper part of the small intestine.

Brief Summary Paragraph Right (33):
Apart from monogastric animals, phospholipase A2 may also be used advantageously in polygastrics. During early lactation of high producing dairy cows for example, it is of interest to include high levels of fat in their diets to compensate in part for the large negative energy balance. From literature it is known that the digestibility of fatty acids in the GI-tract of dairy cows varies as a function of inter alia ration composition and source of fat. Digestibilities of fatty acids have been found to vary between 87% for a diet containing 500 g of saturated fat high in palmitic acid (C16:0) to 64% for a diet containing 1000 g of saturated fat high in stearic acid (C18:0) (Weisbjerg et al., Acta Agric. Scand. Section A, Animal Sciences 42 p. 115-120, 1992).

Brief Summary Paragraph Right (34):
A large part of the variation in digestibility of fatty acids in dairy cows is explained by variations in digestibility in the small intestine (Ibid, p.114-120). Action of phospholipase A2 in the small intestine of dairy cows is likely to enhance the digestibility of fatty acids.

Other Reference Publication (10):
Weisbjert et al., "Digestibility of Fatty Acids in the Gastroinestinal Tract of Dairy Cows Fed with Tallow or Saturated Fats Rich in Stearic Acid or Palmitic Acid" Acta Agric. Scand. Section A, Animal Sciences 42:114-120 (1992).

L8: Entry 30 of 39 File: USPT Aug 17, 1999

DOCUMENT-IDENTIFIER: US 5939303 A
TITLE: Phytases of ruminal microorganisms

Abstract Paragraph Left (1):
Novel phytases derived from ruminal microorganisms are provided. The phytases are capable of catalyzing the release of inorganic phosphorus from phytic acid. Preferred sources of phytases include Selenomonas, Prevotella, Treponema and Megasphaera. A purified and isolated DNA encoding a phytase of Selenomonas ruminantium JY35 (ATCC 55785) is provided. Recombinant expression vectors containing DNA's encoding the novel phytases and host cells transformed with DNA's encoding the novel phytases are also provided. The novel phytases are useful in a wide range of applications involving the dephosphorylation of phytate, including, among other things, use in animal feed supplements.

Brief Summary Paragraph Right (1):
This invention relates to phytases derived from ruminal microorganisms.

Brief Summary Paragraph Right (3):
Production inefficiencies and phosphorus pollution caused by phytate may be effectively addressed by phytase supplementation of diets for monogastric animals. Phytases catalyze the hydrolysis of phytate to myo-inositol and inorganic phosphate, which are then absorbed in the small intestine. In addition to decreasing phosphorus supplementation requirements and reducing the amount of phytate pollutants released, phytases also diminish the antinutritional effects of phytate.

Brief Summary Paragraph Right (4):
Phytases are produced in animal and plant (predominantly seeds) tissues and by a variety of microorganisms (U.S. Pat. No. 3,297,548; Shieh and Ware, 1968; Ware and Shieh, 1967). Despite the array of potential phytase sources, only soil fungi (Aspergillus niger or Aspergillus ficuum) are currently used for commercial production of phytase. The phytase produced by A. ficuum possesses greater specific activity (100 units/mg of protein (wherein units are defined as Imoles of phosphate released per minute)) and thermostability compared to those phytases that have been characterized from other microorganisms (European Patent Application No. 0,420,358 van Gorcum et al., 1991 and U.S. Pat. No. 5,436,156 (van Gorcum et al., issued Jul. 25, 1995)). The A. ficuum phytase is an acid phytase and exhibits little activity above pH 5.5 (Howson and Davis, 1983; van Gorcum et al., 1991). Consequently, activity is limited to a relatively small region of the monogastric digestive tract, in which the pH ranges from 2-3 (in the stomach) to 4-7 (in the small intestine).

Brief Summary Paragraph Right (5):
Although the idea of phytase supplementation of monogastric diets was proposed more than 25 years ago (U.S. Pat. No. 3,297,548, Ware and Shieh, 1967), the high cost of enzyme production has restricted the use of phytase in the livestock industry. In North America, supplemental phytase is generally more expensive than phosphorus supplements. In some circumstances, the cost of phytase utilization may be partially offset if the use of this enzyme also decreases the need for supplementation of a second nutrient such as calcium. The use of phytase in North America is likely to increase as swine and poultry populations increase and as public pressures force a reduction in pollution associated with livestock production. Higher costs of phosphorus supplements and legislation requiring the use of phytase have made the use of this supplement more common in Europe and parts of the Orient than in North America. Governments of the Netherlands, Germany, Korea and Taiwan have enacted or are

enacting legislation to reduce the phosphorus pollution created by monogastric livestock production.

Brief Summary Paragraph Right (6):
A more effective means of increasing phytase utilization is through cost reduction. The cost of phytase can be reduced by decreasing production costs and/or producing an enzyme with superior activity. Recent advances in biotechnology may revolutionize the commercial enzyme industry by offering alternative, cost effective methods of enzyme production. Application of recombinant DNA technology has enabled manufacturers to increase the yields and efficiency of enzyme production, and to create new products. The original source organism need no longer limit the production of commercial enzymes. Genes encoding superior enzymes can be transferred from organisms such as anaerobic bacteria and fungi, typically impractical for commercial production, into well characterized industrial microbial production hosts (e.g., Aspergillus and Bacillus spp.). As well, these genes may be transferred to novel plant and animal expression systems.

Brief Summary Paragraph Right (7):
Unlike monogastric animals, ruminants (e.g., cattle, sheep) readily utilize the phosphorus in phytic acid. It has been demonstrated that phytases are present in the rumen, and it has been proposed that ruminants reared on high grain diets (rich in phytate) do not require dietary phosphorus supplementation due to these ruminal phytases. A single report has attributed this phytase production to ruminal microorganisms (Raun et al., 1956), but overall, the unique capacity of ruminants to utilize phytate has largely been ignored. Raun et al. (1956) prepared microbial suspensions by centrifugal sedimentation (Cheng et al., 1955). Those microbial suspensions were almost certainly contaminated with microscopic particles of plant material. Since plants produce phytases, the study was inconclusive as to whether plant phytases or microbial phytases produced the observed activity. Although Raun et al. have raised the possibility that ruminal phytase production may be attributable to ruminal microorganisms, this possibility has not been explored.

Brief Summary Paragraph Right (8):
In view of the foregoing, there remains a need for low cost phytases having biochemical characteristics well suited for use in animal feed supplements.

Brief Summary Paragraph Right (9):
The inventors have discovered that the rumen is a rich source of microorganisms which produce phytases having biochemical characteristics (such as temperature and pH stability, low metal ion sensitivity and high specific activity) desirable for industrial applications such as animal feed supplementation and inositol production. Ruminal microorganisms tolerate anaerobic conditions and may be either facultative or obligate anaerobes. Ruminal microorganisms may be prokaryotes (i.e., bacteria) or eukaryotes (i.e., fungi, protozoa). As used herein, the term "ruminal microorganisms" includes microorganisms isolated from the digesta or feces of a ruminant animal.

Brief Summary Paragraph Right (10):
Ruminal bacterial species which have been identified as providing particularly active phytases includes Selenomonas ruminantium, Prevotella sp, Treponema bryantii and Megaphaera elsdenii. Prevotella and Selenomonas are Gram negative anaerobic rods from the family Bacteriodaceae.

Brief Summary Paragraph Right (11):
In accordance with the present invention, DNA sequences encoding novel and useful phytases derived from ruminal microorganisms are provided.

Brief Summary Paragraph Right (12):
A phytase gene (phyA) from Selenomonas ruminantium strain JY35 has been cloned and sequenced, and the nucleotide sequence of the phyA gene is provided. The invention extends to DNA sequences which encode phytases and which are capable of hybridizing under stringent conditions with the phyA gene sequence. As used herein, "capable of hybridizing under stringent conditions" means annealing to a subject nucleotide sequence, or its complementary strand, under standard conditions (ie. high temperature and/or low salt content) which tend to disfavor annealing of unrelated sequences. As used herein, "conditions of low stringency" means hybridization and wash conditions of

40-50.degree. C., 6.times.SSC and 0.1% SDS (indicating about 50-80% homology). As used herein, "conditions of medium stringency" means hybridization and wash conditions of 50-65.degree. C., 1.times.SSC and 0.1% SDS (indicating about 80-95% homology). As used herein, "conditions of high stringency" means hybridization and wash conditions of 65-68.degree. C., 0.1.times.SSC and 0.1% SDS (indicating about 95-100% homology).

Brief Summary Paragraph Right (13):
As used herein, the term "phytase" means an enzyme capable of catalyzing the removal of inorganic phosphorus from a myo-inositol phosphate.

Brief Summary Paragraph Right (16):
The invention extends to the S. ruminantium JY35 (ATCC 55785) organism itself, and to methods for identifying and isolating this and other ruminal microorganisms exhibiting phytase activity as well as methods for isolating, cloning and expressing phytase genes from ruminal microorganisms exhibiting phytase activity using part or all of the phyA gene sequence as a probe.

Brief Summary Paragraph Right (17):
The invention further extends to methods for assaying phytase production by a microorganism whereby false positive results caused by microbial acid production are eliminated. Colonies of microorganisms are grown on a growth medium containing phytate. The medium is contacted with an aqueous solution of cobalt chloride. The solution of cobalt chloride is removed and the medium is contacted with aqueous solutions of anmmonium molybdate and ammonium vanadate. After removal of the ammonium molybdate and ammonium vanadate solution, the medium is examined for zones of clearing. False positive results which occur when acid-forming microbes produce zones of clearing are avoided.

Brief Summary Paragraph Right (18):
The invention extends to expression constructs constituting a DNA encoding a phytase of the present invention operably linked to control sequences capable of directing expression of the phytase in a suitable host cell.

Brief Summary Paragraph Right (19):
The invention further extends to host cells which have been transformed with, and express, DNA encoding a phytase of the present invention, and to methods of producing such transformed host cells. As used herein "host cell" includes animal, plant, yeast, fungal, protozoan and prokaryotic host cells.

Brief Summary Paragraph Right (20):
The invention further extends to transgenic plants which have been transformed with a DNA encoding a phytase of the present invention so that the transformed plant is capable of expressing the phytase and to methods of producing such transformed plants. As used herein, "transgenic plant" includes transgenic plants, tissues and cells.

Brief Summary Paragraph Right (21):
Phytases of the present invention are useful in a wide variety of applications involving the dephosphorylation of phytate. Such applications include use in animal feed supplements, feedstuff conditioning, human nutrition, and the production of inositol from phytic acid. Phytases of the present invention may also be used to minimize the adverse effects of phytate metal chelation. The high phytate content of certain feedstuffs such as soy meal decreases their value as protein sources for fish, monogastric animals, young ruminants and infants because the phytate decreases the bioavailability of nutrients by chelating minerals, and binding amino acids and proteins. Treatment of such feedstuffs with the phytases of the present invention will reduce their phytate content by phytase mediated dephosphorylation, rendering the feedstuffs more suitable for use as protein sources. Accordingly, the invention extends to novel feed compositions and feed additives containing a phytase of the present invention. Such feed compositions and supplements may also contain other enzymes, such as, proteases, cellulase, xylanases and acid phosphatases. The phytase may be added directly to an untreated, pelletized, or otherwise processed feedstuff, or it may be provided separately from the feedstuff in, for instance, a mineral block, a pill, a gel formulation, a liquid formulation, or in drinking water. The invention extends to feed inoculant preparations comprising lyophilized microorganisms which express phytases of the present invention under normal growing conditions. With

respect to these feed inoculant preparations, "normal growing conditions" mean culture conditions prior to harvesting and lyophilization of the microorganisms. The microorganisms express phytases during growth of the microbial cultures in large-scale fermenters. The activity of phytases in the microorganisms is preserved by lyophilization of the harvested microbial concentrates containing the phytase.

Brief Summary Paragraph Right (22):
The invention further extends to a method for improving an animal's utilization of dietary phosphate by feeding the animal an effective amount of a phytase of the present invention. As used herein "an effective amount" of a phytase means an amount which results in a statistically significant improvement in phosphorus utilization by the animal. Phytate phosphorus utilization may be evidenced by, for instance, improved animal growth and reduced levels of phytate in animal manure.

Drawing Description Paragraph Right (1):
FIG. 1 is a photograph showing the effect of counterstaining agar medium containing phytate on zones of clearing produced by acid production or phytase activity. Phytate agar was inoculated with S. bovis (top of left petri dish) and S. ruminantium JY35 (bottom of left petri dish) and incubated for 5 d at 37.degree. C. The colonies were scraped off and the medium counterstained with cobalt chloride and ammonium molybdate/ammonium vanadate solutions (right petri plate).

Drawing Description Paragraph Right (2):
FIG. 2 is a graph illustrating the growth (protein) and phytase production of S. ruminantium JY35 in modified Scott and Dehority (1965) broth.

Drawing Description Paragraph Right (3):
FIG. 3 shows transmission electron micrographs of cells from a mid-exponential phase culture of ruminantium JY35 incubated for reaction product deposition by phytase using sodium phytate as the substrate (A, B, C). Untreated control cells are shown for comparison (D, E, F).

Drawing Description Paragraph Right (4):
FIG. 4 is a graph illustrating the phytase pH profile for washed S. ruminantium JY35 cells in five different buffers.

Drawing Description Paragraph Right (7):
FIG. 7 is a graph illustrating the effect of ions (10 mM) on S. ruminantium JY35 phytase activity (Ctr=control).

Drawing Description Paragraph Right (8):
FIG. 8 is a graph illustrating the effect of sodium phytate concentration on S. ruminantium JY35 phytase activity.

Drawing Description Paragraph Right (9):
FIG. 9 is a zymogram developed for confirmation of phytase activity. Concentrates (10.times.) of S. ruminantium JY35 MgCl.sub.2 extract (lanes B-E), low molecular weight markers (lane F, BioRad Laboratories Canada Ltd, Mississauga, Ontario) and A. ficuum phytase (Sigma, 1.6 U, lane A) were resolved by SDS-PAGE in a 10% polyacrylamide gel. Lanes A to E were stained for phytase activity and Lane F was stained with Coomassie brilliant blue.

Drawing Description Paragraph Right (10):
FIG. 10 is a photograph of a phytate hydrolysis plate assay for phytase activities of E. coli DH5.alpha. transformed with pSrP.2 (top), pSrP.2.DELTA.SphI (bottom left), and pSrPf6 (bottom right). Zones of clearing were visible after incubating the plates at 37.degree. C. for 48 h.

Drawing Description Paragraph Right (12):
FIG. 12 is a physical map of pSrP.2. A 2.7-kb fragment, from a Sau3A partial digest of S. ruminatium JY35 genomic DNA, was cloned into the BamHI site of pUC18. This fragment contains the entire gene encoding the phytase from S. ruminatium JY35. The location of a BamHI site lost as a result of the ligation is indicated in square brackets.

Drawing Description Paragraph Right (13):

FIG. 13 is a schematic representation of the deletion analysis of the S. ruminatium phytase gene. The position of phyA is indicated by the horizontal arrow. The hatched boxes indicate segments of the 2.7-kb Sau3A fragment carried by different plasmid derivatives. Phytase activity is indicated in the panel to the right.

Drawing Description Paragraph Right (14):
FIG. 14 is a zymogram developed for phytase activity. E. coli DH5.alpha. (pSrP.2) cells (lane A), E. coli DH5.alpha. (pSrP.2.DELTA.SphI) cells (lane B), and low molecular weight markers (lane C, BioRad Laboratories) were resolved by SDS-PAGE in a 10% polyacrylamide gel. Lanes A and B were stained for phytase activity and Lane C was stained with Coomassie brilliant blue.

Drawing Description Paragraph Right (15):
FIG. 15 is the nucleotide sequence of the S. ruminantium JY35 phytase gene (phyA) (SEQ ID NO. 1) and its deduced amino acid sequence (SEQ ID NO. 2). Nucleotide 1 corresponds to nt 1232 of the 2.7-kb insert of pSrP.2. The putative ribosome binding site is underlined and shown above the sequence as R.B.S. The signal peptidase cleavage site, predicted by the method of von Heijne (1986) is indicated by the .uparw.. The N-terminal amino acid sequence of the phytase secreted by E. coli (pSrPf6) is underlined.

Detailed Description Paragraph Right (1):
The rumen is a complex ecosystem inhabited by more than 300 species of bacteria, fungi and protozoa. Screening these organisms for phytase activity requires the ability to discriminate the phytase activity of individual isolates. This may be accomplished through the assessment of pure cultures from a stock culture collection or separation and cultivation of individual cells through cultural techniques (e.g., streak plate, dilution and micromanipulation). Standard aseptic, anaerobic techniques described for bacteria, fungi and protozoa may be used to accomplish this goal.

Detailed Description Paragraph Right (2):
Suitable enzyme assays are necessary for screening microbial isolates in ruminal fluid samples and from culture collections, and for cloning phytase genes. Assays for measuring phytase activity in solutions have been described in the literature. Sample solutions are typically assayed for phytase activity by measuring the release of inorganic phosphorus (P.sub.i) from phytic acid (Raun et al., 1956; van Hartingsveldt et al., 1993). Phytase activity may also be detected on solid media. Microorganisms expressing phytase produce zones of clearing on agar media containing sodium or calcium phytate (Shieh and Ware, 1968; Howson and Davis, 1983). However, the solid media assays described in the literature were found to be unsatisfactory for screening ruminal bacteria for phytase activity because of the false positive reactions of acid-producing bacteria such as Streptococcus bovis. To overcome this problem, a two-step counterstaining procedure was developed in which petri dishes containing solid medium are flooded first with an aqueous cobalt chloride solution and second with an aqueous ammonium molybdate/ammonium vanadate solution. Following this treatment only clearing zones produced by enzyme activity are evident (FIG. 1).

Detailed Description Paragraph Right (3):
Using the above solutions and solid medium assays, 345 isolates from the Lethbridge Research Centre (Lethbridge, Alberta, Canada) culture collection were screened for phytase activity (Table 1). A total of 29 cultures with substantial phytase activity were identified, including 24 of the genus Selenomonas and 5 of the genus Prevotella. Twelve of these cultures (11 Selenomonas isolates and 1 Prevotella isolate) had phytase activities substantially higher than the other positive cultures (Table 2).

Detailed Description Paragraph Right (4):
The phytase of S. ruminantium JY35 (deposited May 24, 1996 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md., 20852-1776, as ATCC 55785) was selected for further examination and compared to a commercial phytase (Gist-brocades nv, Delft, The Netherlands) from Aspergillus ficuum NRRL 3135 (van Gorcum et al., 1991 and 1995). The phytase of S. ruminantium JY35 (ATCC 55785) is constitutively expressed, exported from the cell and associated with the cell surface. The pH (FIG. 5) and temperature (FIG. 6) profiles of the S. ruminantium JY35 (ATCC 55785) phytase were comparable, if not more suited to industrial production, than are those of the commercial A. ficuum NRRL 3135 phytase. These results demonstrated the

potential of ruminal and anaerobic microbes as sources of phytases with characteristics superior to phytases currently being produced by industry.

Detailed Description Paragraph Right (6):
Using the solid medium phytase assay developed to detect phytase activity produced by ruminal microbes, a S. ruminantium JY35 (ATCC 55785) gene library was screened for positive clones. Of 6000 colonies examined, a single colony was identified as a phytase positive clone by a large zone of clearing around the colony. This clone carried a 5.5-kb plasmid comprising a 2.7-kb Sau3A DNA fragment inserted into cloning vector pUC18. The newly isolated 2.7-kb Sau3A DNA fragment was used as a probe in Southern blot hybridizations. Under high stringency conditions, a discrete band could be detected for S. ruminantium isolate JY35 (ATCC 55785), but not for Prevotella sp. 46/5.sup.2, E. coli DH5.alpha. or A. ficuum NRRL 3135.

Detailed Description Paragraph Right (7):
Plasmid DNA isolated from the newly isolated clone and introduced into E. coli cells by transformation produced ampicillin-resistant, phytase-positive CFUs. Zymogram analysis of cell extracts from E. coli DH5.alpha. cells carrying the 2.7-kb Sau3A DNA fragment from S. ruminantium JY35 (ATCC 55785) revealed a single activity band with an estimated molecular mass of 37 kDa. Deletion and DNA sequence analyses were used to identify the gene (phyA) which encoded the phytase responsible for the activity observed in recombinant E. coli clones. The N-terminal amino acid sequence of the purified 37-kDa phytase expressed in E. coli cells carrying phyA matched the N-terminal amino acid sequence of the mature phytase predicted from the cloned phyA sequence. This indicated conclusively that the nucleotide sequence encoding the phytase had been isolated. The nucleotide sequence and deduced amino acid sequence are shown in FIG. 15.

Detailed Description Paragraph Right (8):
As with other genes, it is possible to use the characterized phytase coding sequence in a variety of expression systems for commercial enzyme production. Application of recombinant DNA technology has enabled enzyme manufacturers to increase the volume and efficiency of enzyme production, and to create new products. The original source organism need no longer limit the production of commercial enzymes. Genes encoding superior enzymes can be transferred from organisms such as anaerobic bacteria and fungi, typically impractical for commercial production, into well characterized industrial microbial production hosts (e.g., Aspergillus, Pichia, Trichoderma, Bacillus spp.). As well, these genes may be transferred to novel plant and animal expression systems.

Detailed Description Paragraph Right (9):
Industrial strains of microorganisms (e.g., Aspergillus niger, Aspergillus ficuum, Aspergillus awamori, Aspergillus oryzae, Trichoderma reesei, Mucor miehei, Kluyveromyces lactis, Pichia pastoris, Saccharomyces cerevisiae, Escherichia coli, Bacillus subtilis or Bacillus licheniformis) or plant hosts (e.g., canola, soybean, corn, potato) may be used to produce phytase. All systems employ a similar approach to gene expression. An expression construct is assembled to include the protein coding sequence of interest and control sequences such as promoters, enhancers and terminators. Other sequences such as signal sequences and selectable markers may also be included. To achieve extracellular expression of phytase, the expression construct of the present invention utilizes a secretory signal sequence. The signal sequence is not included on the expression construct if cytoplasmic expression is desired. The promoter and signal sequence are functional in the host cell and provide for expression and secretion of the coding sequence product. Transcriptional terminators are included to ensure efficient transcription. Ancillary sequences enhancing expression or protein purification may also be included in the expression construct.

Detailed Description Paragraph Right (10):
The protein coding sequences for phytase activity are obtained from ruminal microbial sources. This DNA may be homologous or heterologous to the expression host. Homologous DNA is herein defined as DNA originating from the same species. For example, S. ruminantium may be transformed with DNA from S. ruminantium to improve existing properties without introducing properties that did not exist previously in the species. Heterologous DNA is defined as DNA originating from a different species. For example, the S. ruminatium phyA may be cloned and expressed in E. coli.

Detailed Description Paragraph Right (12):
It is also well-known that often less than a full length protein has the function of the complete protein, for example, a truncated protein lacking an N-terminal, internal or a C-terminal portion often has the biological and/or enzymatic activity of the complete natural protein. Gene truncation experiments involving phyA have confirmed that the truncated protein may retain the function of the intact protein. Escherichia coli clones expressing PhyA missing N-terminal amino acids 1-37 or 1-58 (SEQ ID NO. 2) showed phytase positive phenotypes. In contrast, no phytase activity could be detected for a clone expressing PhyA missing amino acids 307-346 (SEQ ID NO. 2). Those of ordinary skill in the art know how to make truncated protein and proteins with internal deletions. In the present invention, the function of a truncated phytase protein or an internally deleted phytase protein can be readily tested using the assay described hereinbelow and in view of what is generally known in the art.

Detailed Description Paragraph Right (13):
Substituted, internally-deleted and truncated ruminal phytase derivatives which retain substantially the same enzymatic activity as a phytase specifically disclosed herein are considered equivalents of the exemplified phytase and are within the scope of the present invention, particularly where the specific activity of the substituted, internally-deleted or truncated phytase derivative is at least about 10% of the specifically exemplified phytase. The skilled artisan can readily measure the activity of a ruminal phytase, truncated phytase, internally-deleted phytase or substituted phytase using the assay procedures taught herein and in view of what is generally known in the art.

Detailed Description Paragraph Right (14):
This invention includes structurally variant phytases derived from a phytase of a ruminal microorganisms, particularly those derived from a phytase specifically disclosed herein, that are substantially functionally equivalent to that phytase as assayed as described herein in view of what is generally known in the art. Structurally variant, functional equivalents of the phytases of this invention include those phytase of rumina microorganisms having a contiguous amino acid sequence as in the phytase amino acid sequence disclosed herein (SEQ ID NO. 2), particularly those variant phytase which have a contiguous amino acid sequence of a phytase of a rumina microorganism that is a contiguous sequence at least about 25 amino acids in length.

Detailed Description Paragraph Right (15):
The present invention also provides the starting material for the construction of phytases with properties that differ from those of the enzymes isolated herein. The genes can be readily mutated by known procedures (e.g., chemical, site directed, random polymerase chain reaction mutagenesis) thereby creating gene products with altered properties (e.g., temperature or pH optima, specific activity or substrate specificity).

Detailed Description Paragraph Right (30):
Phytase may be extracted from harvested portions or whole plants by grinding, homogenization, and/or chemical treatment. The use of seed specific lipophilic oleosin fusions can facilitate purification by partitioning the oleosin fusion protein in the oil fraction of crushed canola seeds, away from the aqueous proteins (van Rooijen and Moloney, 1994).

Detailed Description Paragraph Right (32):
All or a portion of the microbial cultures and plants may be used directly in applications requiring the action of phytase. Various formulations of the crude or purified phytase preparations may also be prepared. The enzymes can be stabilized through the addition of other proteins (e.g., gelatin, skim milk powder) and chemical agents (e.g., glycerol, polyethylene glycol, reducing agents and aldehydes). Enzyme suspensions can be concentrated (e.g., tangential flow filtration) or dried (spray and drum drying, lyophilization) and formulated as liquids, powders, granules, pills, mineral blocks and gels through known processes. Gelling agents such as gelatin, alginate, collagen, agar, pectin and carrageenan may be used.

Detailed Description Paragraph Right (33):
Further, complete dephosphorylation of phytate may not be achieved by phytase alone.

Phytases may not dephosphorylate the lower myo-inositol phosphates. For instance, an A. ficuum phytase described in U.S. Pat. No. 5,536,156 (van Gorcum et. al., issued Jul. 25, 1995) exhibits low or no phosphatase activity against myo-inositol di-phosphate or myo-inositol mono-phosphate. Addition of another phosphatase, such as an acid phosphatase, to a feed additive of the present invention containing phytase will help dephosphorylate myo-inositol di-phosphate and myo-inositol mono-phosphate.

Detailed Description Paragraph Right (34):
Formulations of the desired product may be used directly in applications requiring the action of a phytase. Liquid concentrates, powders and granules may be added directly to reaction mixtures, fermentations, steeping grains, and milling waste. The formulated phytase can be administered to animals in drinking water, in a mineral block, as a salt, or as a powdered supplement to be sprinkled into feed bunks or mixed with a ration. It may also be mixed with, sprayed on or pelleted with other feed stuffs through known processes. Alternatively, a phytase gene with a suitable promoter-enhancer sequence may be intergrated into an animal genome and selectively expressed in an organ or tissue (e.g. salivary glands, pancreas or epithelial cells) which secrete the phytase enzyme into the gastrointestinal tract, thereby eliminating the need for the addition of supplemental phytase.

Detailed Description Paragraph Right (35):
In a preferred formulation, phytases of the present invention may take the form of microbial feed inoculants. Cultures of microorganisms expressing a native phytase, such as S. ruminantium JY35 (ATCC 55785), or recombinant microorganisms expressing a phytase encoded by a heterologous phytase gene are grown to high concentrations in fermenters and then harvested and concentrated by centrifugation. Food-grade whey and/or other cryoprotective agents are then admixed with the cell concentrate. The resulting mixture is then cryogenically frozen and freeze-dried to preserve phytase activity by standard lyophilization procedures. The freeze-dried culture may be further processed to form a finished product by such further steps as blending the culture with an inert carrier to adjust the strength of the product.

Detailed Description Paragraph Right (36):
All or a portion of the microbial cultures and plants as produced by the present invention may be used in a variety of industrial processes requiring the action of a phytase. Such applications include, without limitation, the manufacture of end products such as inositol phosphate and inositol, production of feed ingredients and feed additives for non-ruminants (e.g., swine, poultry, fish, pet food), in human nutrition, and in other industries (soybean and corn processing, starch, and fermentation) that involve feedstocks containing phytate. Degradation of phytate makes inorganic phosphate and chelated metals available to animals and microorganisms. The action of phytase increases the quality, value and utility of feed ingredients and/or fermentation substrates that are high in phytate. The action of phytases can also accelerate the steeping process and separation processes involved in the wet milling of corn.

Detailed Description Paragraph Right (37):
The phytase genes of the present invention can be used in heterologous hybridization and polymerase chain reaction experiments, directed to isolation of phytase encoding genes from other microorganisms. The examples herein are given by way of illustration and are in no way intended to limit the scope of the present invention. Efforts have been made to ensure the accuracy with respect to numbers used (e.g., temperature, pH, amounts) but the possibility of some experimental variance and deviations should be recognized.

Detailed Description Paragraph Right (38):
Ruminal fluid from a cannulated Holstein cow was collected in a sterile Whirlpak.TM. bag. Fluid may also be withdrawn from the rumen via an orogastric tube. Under a suitable anaerobic atmosphere (e.g., 90% CO.sub.2 and 10% H.sub.2), ten-fold serial dilutions of the rumen fluid were prepared and distributed over the surface of a solid growth medium (e.g., Scott and Dehority, 1965), and the plates were incubated at 39.degree. C. for 18 to 72 h. Isolated colonies were picked with a sterile loop and the cells were spread over the surface of fresh agar medium to produce isolated colonies. The cells from a single colony were confirmed by morphological examination to represent a pure culture and were cultured and stored in the Lethbridge Research

Centre ("LRC") culture collection or used as a source of enzymatic activity or genetic material.

Detailed Description Paragraph Right (39):
A. Phytase assays

Detailed Description Paragraph Right (40):
Sample solutions (culture filtrates, cell suspensions, lysates, washes or distilled water blanks) were assayed for phytase activity by incubating 150 .mu.l of the solution with 600 .mu.l of substrate solution [0.2% (w/v) sodium phytate in 0.1M sodium acetate buffer, pH 5.0] for 30 min at 37.degree. C. The reaction was stopped by adding 750 .mu.l of 5% (w/v) trichloroacetic acid. Released orthophosphate in the reaction mixture was measured by the method of Fiske and Subbarow (1925). Freshly prepared colour reagent [750 .mu.l of a solution containing 4 volumes of 1.5% (w/v) ammonium molybdate in a 5.5% (v/v) sulfuric acid solution and 1 volume of a 2.7% (w/v) ferrous sulfate solution] was added to the reaction mixture and the production of phosphomolybdate was measured spectrophotometrically at 700 nm. Results were compared to a standard curve prepared with inorganic phosphate. One unit ("Unit") of phytase was defined as the amount of enzyme required to release one .mu.mole of inorganic phosphate (P.sub.i) per min under the assay conditions.

Detailed Description Paragraph Right (41):
An improved phytase plate assay was developed which eliminated false positive results caused by microbial acid production. Bacterial isolates were grown under anaerobic conditions on modified Scott and Dehority (1965) agar medium containing 5% (v/v) rumen fluid, 1.8% (w/v) agar and 2.0% (w/v) sodium phytate for 5 d at 37.degree. C. Colonies were washed from the agar surface and the petri plates were flooded with a 2% (w/v) aqueous cobalt chloride solution. After a 5-min incubation at room temperature the cobalt chloride solution was replaced with a freshly prepared solution containing equal volumes of a 6.25% (w/v) aqueous ammonium molybdate solution and 0.42% (w/v) ammonium vanadate solution. Following a 5-min incubation, the ammonium molybdate solution/ammonium vanadate solution was removed and the plates examined for zones of clearing. The effectiveness of this counterstaining technique is demonstrated in FIG. 1. Prior to staining, zones of clearing were evident around colonies of phytase-producing S. ruminantium JY35 (ATCC 55785) and lactic acid-producing S. bovis grown on agar medium containing phytate (FIG. 1, left petri plate). The false positive zones of clearing resulting from acid production by S. bovis colonies were eliminated by counterstaining the plates with cobalt chloride and ammonium molybdate/ammonium vanadate solutions (FIG. 1, right petri plate).

Detailed Description Paragraph Right (42):
B. Phytase activity of ruminal bacteria

Detailed Description Paragraph Right (43):
The phytase activities of 345 rumen bacteria from the LRC culture collection were determined (Table 1). The anaerobic technique of Hungate (1950), as modified by Bryant and Burkey (1953), or an anaerobic chamber with a 90% CO.sub.2 and 10% H2 atmosphere was used to cultivate the microorganisms in the LRC culture collection. Phytase screening was performed on isolates grown anaerobically (100% CO.sub.2) in Hungate tubes with 5 mL of modified Scott and Dehority medium (1965) containing 5% (v/v) rumen fluid, 0.2% (w/v) glucose, 0.2% (w/v) cellobiose and 0.3% (w/v) starch. After 18 to 24 h incubation at 39.degree. C., whole cells or culture supernatants were assayed for phytase activity. Selenomonads were the predominant phytase producers (93% of the isolates tested had phytase activity, Table 1). Prevotella was the only other genus from which a significant number of positive cultures was identified (11 phytase positive isolates out of 40 tested). A total of 29 cultures with substantial phytase activity were identified. These included 24 of the genus Selenomonas and 5 of the genus Prevotella. Twelve of these cultures (11 Selenomonas and 1 Prevotella isolate) had phytase activities substantially higher than the other positive cultures (Table 2). In all instances, the phytase activity was predominantly cell associated.

Detailed Description Paragraph Right (44):
A. Growth and phytase production

Detailed Description Paragraph Right (45):

Phytase production during growth of S. ruminantium JY35 (ATCC 55785) was examined. S. ruminantium JY35 (ATCC 55785) was grown at 39.degree. C. in Hungate tubes with 5 mL of modified Scott and Dehority broth (1965) containing 5% (v/v) ruminal fluid. Growth (protein concentration) and phytase activity (cell associated) were monitored at intervals over a 24-h time period. Maximal growth and phytase activity of S. ruminantium JY35 (ATCC 55785) were achieved 8-10 h after inoculation (FIG. 2). Cell growth was mirrored by increases in phytase activity.

Detailed Description Paragraph Right (46):
B. Localization of phytase activity

Detailed Description Paragraph Right (47):
S. ruminantium JY35 (ATCC 55785) phytase activity was determined to be predominantly cell associated. Little phytase activity was detected in culture supernatants and cell washes. The phytase activity of S. ruminantium JY35 (ATCC 55785) was localized by electron microscopy as described by Cheng and Costerton (1973). Cells were harvested by centrifugation, washed with buffer, embedded in 4% (w/v) agar, prefixed in 0.5% glutaraldehyde solution for 30 min and fixed for 2 hours in 5% (v/v) glutaraldehyde solution. Samples were washed five times with cacodylate buffer (0.1M, pH 7.2) and treated with 2% (w/v) osmium tetroxide, washed five times with cacodylate buffer, dehydrated in a graded ethanol series, and embedded in Spurr's resin (J. B. EM Services Inc.). Ultrathin sections were cut with a Reichert model OM U3 ultramicrotome and stained with 2% (w/v) uranyl acetate and lead citrate. Specimens were viewed with Hitachi H-500 TEM at an accelerating voltage of 75 kV. A comparison of S. ruminantium JY35 (ATCC 55785) cells incubated with substrate for reaction product deposition with untreated cells clearly indicated that the phytase activity was associated with the cell outer membrane surfaces (FIG. 3). Deposition of electron dense material on the outer cell surfaces of treated cells was the result of phytase activity (FIGS. 3A, B and C).

Detailed Description Paragraph Right (48):
C. Phytase pH optimum

Detailed Description Paragraph Right (49):
Initial determinations of the pH optimum of the S. ruminantium JY35 (ATCC 55785) phytase were conducted with whole cells. Phytase activity was optimal over a pH range of 4.0 to 5.5 (FIG. 4). A second pH curve was generated with a MgCl.sub.2 cell extract (FIG. 5). Cells from a 100-mL overnight culture were washed twice with sterile distilled water, resuspended in 0.3 volumes of a 0.2M MgCl.sub.2 aqueous solution and incubated overnight at 0.degree. C. The solution was clarified by centrifugation and the resulting extract was used in phytase assays. Four buffers systems were used to cover the pH range; glycine (pH 1.5-3.0), formate (pH 3.0-4.0), acetate (pH 4.0-5.5) and succinate (pH 5.5-6.5).

Detailed Description Paragraph Right (50):
D. Phytase temperature optimum The temperature optimum of the S. ruminantium JY35 (ATCC 55785) phytase activity was determined at pH 5.0 (0.1M sodium acetate buffer) with MgCl.sub.2 cell extract. The enzyme retained over 50% of its activity over a temperature range of 37 to 55.degree. C. (FIG. 6).

Detailed Description Paragraph Right (51):
E. The effect of ions and substrate concentration on phytase activity

Detailed Description Paragraph Right (52):
The effect of various ions (10 mM) and substrate concentration on whole cell phytase activity were determined at pH 5.0 (0.1M sodium acetate buffer). Phytase activity was stimulated by the addition of Ca.sup.++, Na.sup.+, K.sup.+ and Mg.sup.++, inhibited by Fe.sup.++, Zn.sup.++ and Mn.sup.++ and unaffected by Co.sup.++ and Ni.sup.++ (FIG. 7). The effect of substrate concentration on phytase activity in a S. ruminantium JY35 (ATCC 55785) MgCl.sub.2 cell extract is presented in FIG. 8.

Detailed Description Paragraph Right (54):
The molecular size of the phytase in S. ruminantium JY35 (ATCC 55785) was determined by zymogram analysis. A ten-fold concentrated crude MgCl.sub.2 released extract was mixed with 20 .mu.L of sample loading buffer (Laemnmli, 1970) in a microtube and the

microtube was placed in a boiling water bath for 5 minutes. The denatured MgCl.sub.2 extracts were resolved by SDS-PAGE on a 10% separating gel topped with a 4% stacking gel (Laemmli, 1970). Following electrophoresis, the phytase was renatured by soaking the gel in 1% Triton X-100 for 1 h at room temperature and 0.1M sodium acetate buffer (pH 5.0) for 1 h at 4.degree. C. Phytase activity was detected by incubating the gel for 16 h in a 0.1 M sodium acetate buffer (pH 5.0) containing 0.4% sodium phytate. The gel was treated with the cobalt chloride and ammonium molybdate/ammonium vanadate staining procedure described for the phytase plate assays in Example 2. A single dominant activity band, corresponding to a molecular mass of approximately 35 to 45 kDa, was observed (FIG. 9).

Detailed Description Paragraph Right (55):
A. Isolation of phytase positive Escherichia coli clone

Detailed Description Paragraph Right (56):
Genomic DNA libraries were prepared for S. ruminantium JY35 (ATCC 55785) according to published procedures (Hu et al., 1991; Sambrook et al., 1989). Genomic DNA was extracted from a fresh overnight culture of S. ruminantium JY35 (ATCC 55785) using a modification of the protocol described by Priefer et al. (1984). S. ruminantium JY35 (ATCC 55785) genomic DNA was partially digested with Sau3A and gel purified to produce DNA fragments in the 2- to 10-kb range. A genomic library was constructed by ligating BamHI-digested, dephosphorylated pUC18 with S. ruminantium JY35 (ATCC 55785) Sau3A genomic DNA fragments. Escherichia coli DH5.alpha. competent cells (Gibco BRL, Mississauga, ON) were transformed with the ligation mix and 6,000 clones carrying inserts were screened for phytase activity (zones of clearing) on LB phytase screening agar [LB medium, 1.0% sodium phytate (filter sterilized), 100 mM HEPES (pH 6.0-6.5), and 0.2% CaCl.sub.2] containing ampicillin (100 .mu.g/mL). A phytase-positive clone SrP.2 was isolated and phytase activity confirmed through enzyme assays (FIG. 10). Very high levels of phytase activity were found in the medium as well as associated with the E. coli cells (Table 3). Plasmid DNA isolated from clone SrP.2 carried a 5.5-kb plasmid, designated pSrP.2, consisting of pUC18 containing a 2.7-kb Sau3A insert.

Detailed Description Paragraph Right (60):
A. Evidence for the cloning of a phytase gene

Detailed Description Paragraph Right (61):
Escherichia coli DH5.alpha. competent cells (Gibco BRL, Mississauga, ON) were transformed with plasmids pUC18 and pSrP.2. The resulting ampicillin-resistant transformants were tested for phytase activity on LB phytase screening agar. Only E. coli DH5.alpha. cells transformed with pSrP.2 produced clearing zones on LB phytase screening agar.

Detailed Description Paragraph Right (63):
The phytase gene was localized on the 2.7-kb Sau3A insert by restriction endonuclease and deletion analyses (Ausubel et al., 1990; Sambrook et al., 1989). Cells carrying plasmid pSrP.2.DELTA.SphI, constructed by the deletion of the 1.4-kb SphI fragment from pSrP.2, lacked phytase activity (FIG. 12 and FIG. 13, Table 3).

Detailed Description Paragraph Right (65):
The molecular mass of the phytase produced by E. coli DH5.alpha. (pSrP.2) was determined by zymogram analysis. One mL of an overnight culture was transferred to a 1.5-mL microtube. The cells were harvested by centrifugation and washed with 0.1M sodium acetate buffer (pH 5.5). The cell pellet was resuspended in 80 .mu.L of sample loading buffer (Laemmli, 1970) and the microtube was placed in a boiling water bath for 5 minutes. The resulting cell extracts were resolved by SDS-PAGE on a 10% separating gel topped with a 4% stacking gel (Laemmli, 1970) and the gel was stained for phytase activity as described in Example 3F. A single dominant activity band, corresponding to a molecular mass of approximately 37 kDa, was observed (FIG. 14, lane A). A corresponding activity band was not observed for E. coli DH5.alpha. (pSrP.2.DELTA.SphI) cells (FIG. 14, lane B).

Detailed Description Paragraph Right (68):
The sequence of the 2.7-kb DNA insert was determined and DNA structural analysis identified an open reading frame (ORF2; bp1493 to 2504) overlapping the SphI site of

the 2.7-kb Sau3A insert and large enough to encode the 37 kDa phytase. Phytase activity was eliminated by deleting bp 1518 through to the end of the 2.7-kb Sau3A fragment (pSrPr6, Table 3, FIG. 13). This was accomplished by cloning the PCR product of pSrP.2 bounded by sequencing primer SrPr6 (CGG GAT GCT TCT GCC AGT AT, SEQ ID NO. 3 the reverse complement of bp 1518 to 1538) and M13 Forward primer (CGC CAG GGT TTT CCC AGT CAC GAC) into pGEM-T (Promega Corp.). A PCR product subclone (pSrPf6) of pSrP.2, bounded by primer SrPf6 (bp 1232 to 1252, CGT CCA CGG AGT CAC CCT AC) SEQ ID NO. 4 and M13 Reverse primer (AGC GGA TAA CAA TTT CAC ACA GGA), and containing ORF2 plus 252 bp upstream of the SphI cleavage site retained phytase activity (Table 3, FIG. 13).

Detailed Description Paragraph Right (69):
The sequence and translation of the S. ruminatium phytase gene (phyA) is shown in FIG. 15. Translation of ORF2 would result in the expression of a 346-amino acid polypeptide with a predicted molecular weight of 39.6 kDa (FIG. 15). The first 31 residues were typical of a prokaryote signal sequence, encompassing a basic N-terminus and central hydrophobic core (von Heijne, 1986). Application of the method of von Heijne (1986) predicted the signal peptidase cleavage site most probably occurs before Ala.sup.28 or Pro.sup.31. This was confirmed by determining the N-terminal amino acid sequence of gel purified from E. coli DH5.alpha. (pSrPf6) culture supernatant (FIG. 15). The secreted mature protein has a putative mass of 36.5 kDa.

Detailed Description Paragraph Right (70):
A comparison of the phyA amino acid sequence with known protein sequences from the MasDNASIS SWISSPROT database revealed no significant similarities to any published sequences including Aspergillus niger phytase genes phyA and phyB.

Detailed Description Paragraph Right (71):
Cell free supernatants, prepared from overnight cultures of E. coli (pSrPf6), were mixed 3:1 (v/v) with Ni.sup.++ -NTA agarose pre-equilibrated in 0.1M Tris (pH 7.9), 0.3M NaCl buffer. The mixture was incubated at room temperature for 0.5 h and washed 3.times.with 0.1M Tris (pH 7.9), 0.3M NaCl buffer. The phytase activity was eluted from the resin with 1 volume 0.1M sodium acetate (pH 5.0), 0.3M NaCl. When resolved on SDS-polyacrylamide gels stained with Coomassie brilliant blue, over 70% of the eluted protein formed a single 37-kDa protein band. Zymogram and N-terminal amino acid sequence analyses confirmed that the 37-kDa band corresponded to the phytase encoded by the cloned S. ruminantium JY35 (ATCC 55785) phyA. The specific activity of Ni.sup.++ -NTA agarose-purified phytase ranged from 200 to 400 .mu.mol phosphate released/min/mg protein. This is 2 to 4 times higher than the specific activity reported for the purified A. ficuum NRRL 3135 phytase (van Gorcum et al., 1991, 1995; van Hartingsveldt et al., 1993).

Detailed Description Paragraph Right (72):
Isolation and characterization of phyA from S. ruminantium JY35 (ATCC 55785) enables the large scale production of protein PhyA in any of a number of prokaryotic (e.g., E. coli and B. subtilis) or eukaryotic (e.g., fungal--Pichia, Saccharomyces, Aspergillus, Trichoderma; plant--Brassica, Zea, Solanum; or animal--poultry, swine or fish) expression systems using known methods. Teachings for the construction and expression of phyA in E. coli , P. pastoris, and B. napus are provided below. Similar approaches may be adopted for expression of the S. ruminantium JY35 (ATCC 55785) phytase in other prokaryotic and eukaryotic organisms.

Detailed Description Paragraph Right (78):
Expression of the S. ruminantium JY35 (ATCC 55785) phytase by transformed E. coli cells is tested by growing the cells under vigorous aeration at 37.degree. C. in a suitable liquid medium (e.g., LB or 2.times.YT) containing the appropriate antibiotic selection until the optical density (at 600 nm) is between 0.5 and 1.0. The tac promoter is induced by adding isopropyl-.beta.-D-thiogalactoside (IPTG) to a final concentration between 0.1 and 2 mM. The cells are cultivated for an additional 2 to 4 h and harvested by centrifugation. Protein expression is monitored by SDS-PAGE, and western blot/immunodetection techniques. The expressed PhyA may be extracted by breaking (e.g., sonication or mechanical disruption) the E. coli cells. Protein inclusions of PhyA may be harvested by centrifugation and solubilized with 1 to 2% SDS. The SDS may be removed by dialysis, electroelution or ultrafiltration. The phytase activity of prepared cell extracts may be assayed by standard methods described in Example 2.

Detailed Description Paragraph Right (79):
An expression construct is constructed in which the region encoding the mature PhyA is translationally fused with the secretion signal sequences found on P. pastoris expression vectors (Pichia Expression Kit Instruction Manual, Invitrogen Corporation, San Diego, Calif.) in order to express the S. ruminantium phytase as a secreted product. The promoter and secretion signal sequences may be replaced by those from other promoters that provide for efficient expression in Pichia. The expression construct is introduced into P. pastoris cells by transformation.

Detailed Description Paragraph Right (84):
Expression of the S. ruminantium JY35 (ATCC 55785) phytase by transformed P. pastoris cells is tested by growing the cells at 30.degree. C. under vigorous aeration in a suitable liquid medium (e.g. buffered complex glycerol medium such as BMGY) until a culture optical density (at 600 nm) (OD.sub.600) of 2 to 6 is reached. The cells are harvested and resuspended to an OD.sub.600 of 1.0 in an inducing medium (e.g., buffered complex methanol medium, BMMY) and incubated for a further 3 to 5 days. Cells and cell-free culture supernatant are collected and protein expression is monitored by enzyme assay, SDS-PAGE, and western blot/immunodetection techniques.

Detailed Description Paragraph Right (85):
Transformation and gene expression methods have been developed for a wide variety of monocotyledonous and dicotyledonous crop species. In this example, a S. ruminantium JY35 (ATCC 55785) phytase expression construct is constructed in which the region encoding the mature PhyA is translationally fused with an oleosin coding sequence in order to target seed oil body specific expression of the S. ruminantium phytase. The promoter and/or secretion signal sequences may be replaced by those from other promoters that provide for efficient expression in B. napus or other transformable plant species. The expression construct is introduced into B. napus cells by Agrobacterium-mediated transformation.

Detailed Description Paragraph Right (89):
Transgenic B. napus is prepared as described by van Rooijen and Moloney (1994). Agrobacterium tumefaciens strain EHA101 is transformed by electroporation with pCGOBPphyA. Cotyledonary petioles of B. napus are transformed with A. tumefaciens EHA101 (pCGOBPphyA). Transgenic plants are regenerated from explants that root on hormone-free MS medium containing 20 .mu.g/mL kanamycin. Young plants are assayed for NPTII activity, grown to maturity and allowed to self pollenate and set seed. Seeds from individual transformants are pooled and part of the seed sample is assayed for the presence of phytase activity and compared to seeds from untransformed plants. Second generation plants (T2) are propagated from the seeds of clones with the highest levels of phytase activity. Seeds from the T2 plants homozygous for NPTII (hence also for phyA) are selected and used for mass propagation of plants (T3) capable of producing the highest amounts of phytase.

Detailed Description Paragraph Right (90):
To identify a phytase gene related to phyA, hybridization analysis can be used to screen nucleic acids from one or more ruminal isolates of interest using phyA (SEQ ID NO. 1) or portions thereof as probes by known techniques (Sambrook, 1989; Ausubel, 1990) as described in example 4B. Related nucleic acids may be cloned by employing known techniques. Radioisotopes (i.e., .sup.32 P) may be required when screening organisms with complex genomes in order to increase the sensitivity of the analysis. Polymerase chain reaction (PCR) amplification may also be used to identify genes related to phyA. Related sequences found in pure or mixed cultures are preferentially amplified by PCR (and variations of such as Reverse Transcription--PCR) with oligonucleotides primers designed using SEQ ID NO. 1. Amplified products may be visualized by agarose gel electrophoresis and cloned using known techniques. A variety of materials, including cells, colonies, plaques, and extracted nucleic acids (e.g., DNA, RNA), may be examined by these techniques for the presence of related sequences. Alternatively, known immunodetection techniques employing antibodies specific to PhyA (SEQ ID NO. 2) can be used to screen whole cells or extracted proteins of interest for the presence of related phytase(s).

Detailed Description Paragraph Left (2):
Screening ruminal bacteria for phytase activity

Detailed Description Paragraph Left (3):
Phytase activity of Selenomonas ruminantium JY35 (ATCC 55785)

Detailed Description Paragraph Left (4):
Cloning of a phytase gene (phyA) from Selenomonas ruminantium JY35 (ATCC 55785)

Detailed Description Paragraph Left (5):
Characterization of Selenomonas ruminantium phytase gene

Detailed Description Paragraph Left (11):
Identification of Related Phytase Genes in Other Microorganisms

Detailed Description Paragraph Type 0 (18):
Shieh, T. R. and J. H. Ware. 1968. Survey of microorganisms for the production of extracellular phytase. Appl. Microbiol. 16:1348-1351.

Detailed Description Paragraph Type 0 (20):
R. F .M. van Gorcom, and C. A. M. J Van Den Hondel. 1993. Cloning, characterization and overexpression of the phytase gene (phyA) of Aspergillus niger. Gene 127:87-94.

Detailed Description Paragraph Table (1):
TABLE 1 Phytase activity among rumen bacteria Phytase Activity isolates tested Microorganism Number of Very Strong Prevotella sp. 1 Selenomonas ruminantium 11 Strong Prevotella ruminicola 4 S. ruminantium 13 Moderate Bacillus sp. 1 Megasphaera elsdenii 7 P. ruminicola 6 S. ruminantium 37 Treponema sp. 1 Negative Anaerovibrio lipolytica 2 Bacillus sp. 4 Butyrivibrio fibrisolvens 47 Clostridium sp. 1 Coprococcus sp. 3 Enterococcus sp. 4 Eubacterium sp. 7 Fibrobacter succinogenes 8 Fusobacterium sp. 3 Lachnospira multiparus 4 Lactobacillus sp. 20 M. elsdenii 7 Peptostreptococcus sp. 1 P. ruminicola 41 Ruminobacter amylophilus 4 Ruminococcus albus 7 Ruminococcus flavefaciens 10 S. ruminantium 4 Streptococcus bovis 48 Streptococcus milleri 1 Staphylococcus sp. 6 Succinovibrio dextrisolvens 12 Treponema sp. 12 Unknown 8 Total isolates screened 345

Detailed Description Paragraph Table (2):
TABLE 2 Phytase activity of selected rumen bacterial isolates Phytase activity Isolate (mu*/mL) Selenomonas ruminantium JY35 646 Selenomonas ruminantium KJ118 485 Selenomonas ruminantium BS131 460 Selenomonas ruminantium HD141 361 Selenomonas ruminantium HD86 286 Selenomonas ruminantium JY135 215 Selenomonas ruminantium D 69 Selenomonas ruminantium HD16 52 Selenomonas ruminantium BS114 47 Selenomonas ruminantium JY4 27 Prevotella sp. 46/5.sup.2 321 Prevotella ruminicola JY97 68 Prevotella ruminicola KJ182 61 Prevotella ruminicola JY106 49 Megasphaera elsdenii JY91 5 *U = .mu.moles, P.sub.i released/min

Detailed Description Paragraph Table (3):
TABLE 3 Overexpression of S. ruminantium phytase in recombinant E. coli DH5.alpha. Specific Activity Sample (Units/ Strain Composition Units.sup.1 /mL mg protein) E. coli (pSrP.2) cells .sup. 0.30 (0.08).sup.2 1.56 (0.41) supernatant 0.308 (0.21) 2.64 (1.51) E. coli (pSrPf6) cells 0.91 (0.41) 6.42 (0.64) supernatant 5.10 (0.58) 22.83 (1.67) E. coli (pSrP.2 SphI) cells .sup. ND.sup.3 ND supernatant ND ND .sup.1 Units = .mu.moles P.sub.i released/min .sup.2 Numbers in parenthese are standard errors .sup.3 ND = not detected

Detailed Description Paragraph Table (4):
SEQUENCE LISTING - (1) GENERAL INFORMATION: - (iii) NUMBER OF SEQUENCES: 7 - (2) INFORMATION FOR SEQ ID NO:1: - (i) SEQUENCE CHARACTERISTICS: #pairs (A) LENGTH: 1401 base (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: circular - (ii) MOLECULE TYPE: DNA (genomic) - (iii) HYPOTHETICAL: NO - (iv) ANTI-SENSE: NO - (vi) ORIGINAL SOURCE: #ruminantium) ORGANISM: Selenomonas (B) STRAIN: JY35 - (vii) IMMEDIATE SOURCE: (A) LIBRARY: Genomic DN - #A library (B) CLONE: pSrP.2 - (ix) FEATURE: (A) NAME/KEY:

CDS (B) LOCATION: 231..1268 (C) IDENTIFICATION METHOD: - # experimental #/codon.sub.-- start= 231MATION: /function=- # "Dephosphorylation of phytic acid" /product=- # "Phytase" /evidence=- # EXPERIMENTAL #"phyA" /gene= #1 /number= /standard.sub.-- - #name= "myo-inositol hexaphosphate phosphohydro - #lase" /citation=- # ([1]) - (ix) FEATURE: (A) NAME/KEY: sig.sub.-- - #peptide (B) LOCATION: 231..311 (C) IDENTIFICATION METHOD: - # experimental #/codon.sub.-- start= 1ORMATION: /function=- # "phytase secretion" /product=- # "Signal peptide" /evidence=- # EXPERIMENTAL /citation=- # ([1]) - (ix) FEATURE: (A) NAME/KEY: mat.sub.-- - #peptide (B) LOCATION: 312..1268 (C) IDENTIFICATION METHOD: - # experimental #/codon.sub.-- start= 312MATION: /product=- # "Phytase" /evidence=- # EXPERIMENTAL #2 /number= /citation=- # ([1]) - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1: - CGTCCACGGA GTCACCCTAC TATACGACGT ATGTGAAGTT CACGTCGAAG TT - #CTAGGGAA 60 - TCACCGATTC GTGCAGGATT TTACCACTTC CTGTTGAAGC GGATGAGAAG GG - #GAACCGCG 120 - AAGCGGTGGA AGAGGTGCTG CACGACGGAC GATCGCGCTG AATGAATCAG TG - #CTTCCTAA 180 - CTATTGGGAT TCCGCGCAGA CGCGCGGATG GAGTAAAGGA GTAAGTTGTT AT - #G AAA 236 # Met Lys 27 - TAC TGG CAG AAG CAT GCC GTT CTT TGT AGT CT - #C TTG GTC GGC GCA TCC 284 Tyr Trp Gln Lys His Ala Val Leu Cys Ser Le - #u Leu Val Gly Ala Ser - #10 - CTC TGG ATA CTG CCG CAG GCC GAT GCG GCC AA - #G GCG CCG GAG CAG ACG 332 Leu Trp Ile Leu Pro Gln Ala Asp Ala Ala Ly - #s Ala Pro Glu Gln Thr #1 5 - GTG ACG GAG CCC GTT GGG AGC TAC GCG CGC GC - #G GAG CGG CCG CAG GAC 380 Val Thr Glu Pro Val Gly Ser Tyr Ala Arg Al - #a Glu Arg Pro Gln Asp # 20 - TTC GAG GGC TTT GTC TGG CGC CTC GAC AAC GA - #C GGC AAG GAG GCG TTG 428 Phe Glu Gly Phe Val Trp Arg Leu Asp Asn As - #p Gly Lys Glu Ala Leu # 35 - CCG CGT AAT TTC CGC ACG TCG GCT GAC GCG CT - #G CGC GCG CCG GAG AAG 476 Pro Arg Asn Phe Arg Thr Ser Ala Asp Ala Le - #u Arg Ala Pro Glu Lys # 55 - AAA TTC CAT CTC GAC GCC GCG TAT GTA CCG TC - #G CGC GAG GGC ATG GAT 524 Lys Phe His Leu Asp Ala Ala Tyr Val Pro Se - #r Arg Glu Gly Met Asp # 70 - GCA CTC CAT ATC TCG GGC AGT TCC GCA TTC AC - #G CCG GCG CAG CTC AAG 572 Ala Leu His Ile Ser Gly Ser Ser Ala Phe Th - #r Pro Ala Gln Leu Lys # 85 - AAC GTT GCC GCG AAG CTG CGG GAG AAG ACG GC - #T GGC CCC ATC TAC GAT 620 Asn Val Ala Ala Lys Leu Arg Glu Lys Thr Al - #a Gly Pro Ile Tyr Asp # 100 - GTC GAC CTA CGG CAG GAG TCG CAC GGC TAT CT - #C GAC GGT ATC CCC GTG 668 Val Asp Leu Arg Gln Glu Ser His Gly Tyr Le - #u Asp Gly Ile Pro Val # 115 - AGC TGG TAC GGC GAG CGC GAC TGG GCA AAT CT - #C GGC AAG AGC CAG CAT 716 Ser Trp Tyr Gly Glu Arg Asp Trp Ala Asn Le - #u Gly Lys Ser Gln His 120 1 - #25 1 - #30 1 - #35 - GAG GCG CTC GCC GAC GAG CGG CAC CGC TTG CA - #C GCA GCG CTC CAT AAG 764 Glu Ala Leu Ala Asp Glu Arg His Arg Leu Hi - #s Ala Ala Leu His Lys # 150 - ACG GTC TAC ATC GCG CCG CTC GGC AAG CAC AA - #G CTC CCC GAG GGC GGC 812 Thr Val Tyr Ile Ala Pro Leu Gly Lys His Ly - #s Leu Pro Glu Gly Gly # 165 - GAA GTC CGC CGC GTA CAG AAG GTG CAG ACG GA - #A CAG GAA GTC GCC GAG 860 Glu Val Arg Arg Val Gln Lys Val Gln Thr Gl - #u Gln Glu Val Ala Glu # 180 - GCC GCG GGG ATG CGC TAT TTC CGC ATC GCG GC - #G ACG GAT CAT GTC TGG 908 Ala Ala Gly Met Arg Tyr Phe Arg Ile Ala Al - #a Thr Asp His Val Trp # 195 - CCA ACG CCG GAG AAC ATC GAC CGC TTC CTC GC - #G TTT TAC CGC ACG CTG 956 Pro Thr Pro Glu Asn Ile Asp Arg Phe Leu Al - #a Phe Tyr Arg Thr Leu 200 2 - #05 2 - #10 2 - #15 - CCG CAG GAT GCG TGG CTC CAT TTC CAT TGT GA - #A GCC GGT GTC GGC CGC 1004 Pro Gln Asp Ala Trp Leu His Phe His Cys Gl - #u Ala Gly Val Gly Arg # 230 - ACG ACG GCG TTC ATG GTC ATG ACG GAT ATG CT - #G AAG AAC CCG TCC GTA 1052 Thr Thr Ala Phe Met Val Met Thr Asp Met Le - #u Lys Asn Pro Ser Val # 245 - TCG CTC AAG GAC ATC CTC TAT CGC CAG CAC GA - #G ATC GGC GGC TTT TAC 1100 Ser Leu Lys Asp Ile Leu Tyr Arg Gln His Gl - #u Ile Gly Gly Phe Tyr # 260 - TAC GGG GAG TTC CCC ATC AAG ACG AAG GAT AA - #A GAT AGC TGG AAG ACG 1148 Tyr Gly Glu Phe Pro Ile Lys Thr Lys Asp Ly - #s Asp Ser Trp Lys Thr # 275 - AAA TAT TAT AGG GAA AAG ATC GTG ATG ATC GA - #G CAG TTC TAC CGC TAT 1196 Lys Tyr Tyr Arg Glu Lys Ile Val Met Ile Gl - #u Gln Phe Tyr Arg Tyr 280 2 - #85 2 - #90 2 - #95 - GTG CAG GAG AAC CGC GCG GAT GGC TAC CAG AC - #G CCG TGG TCG GTC TGG 1244 Val Gln Glu Asn Arg Ala Asp Gly Tyr Gln Th - #r Pro Trp Ser Val Trp # 310 - CTC AAG AGC CAT CCG GCG AAG GCG TAAAAGCGCA GG - #CGGCGGCT CGGAGTCAGG 1298 Leu Lys Ser His Pro Ala Lys Ala 315 - GAAATGGCGC TGCCAGCACG GGACGCGCGG CGGCGGATGC TGCGCCGGTC AG - #GGATGATT 1358 # 140 - #1ATGGTTTT ATGAGGTGGA TCC - (2) INFORMATION FOR SEQ ID NO:2: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 346 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: protein - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: - Met Lys Tyr Trp Gln Lys His Ala Val Leu Cy - #s Ser Leu Leu Val Gly 15 - Ala Ser Leu Trp Ile Leu Pro Gln Ala Asp Al - #a Ala Lys Ala Pro Glu # 5 1 - Gln Thr Val Thr Glu Pro Val Gly Ser Tyr Al - #a Arg Ala Glu Arg Pro # 20 - Gln Asp Phe Glu Gly Phe Val Trp Arg Leu As - #p Asn Asp Gly Lys Glu # 35 - Ala Leu Pro Arg Asn Phe Arg Thr Ser Ala As - #p Ala Leu Arg Ala Pro # 50 - Glu Lys Lys Phe His Leu Asp Ala Ala Tyr Va - #l Pro Ser Arg Glu Gly # 65 - Met Asp Ala Leu His Ile Ser Gly Ser Ser Al - #a Phe Thr Pro Ala Gln # 85 - Leu Lys Asn Val Ala Ala Lys Leu Arg Glu Ly - #s Thr Ala Gly Pro Ile # 100 - Tyr Asp Val Asp Leu Arg Gln Glu Ser His Gl - #y Tyr Leu Asp Gly Ile # 115 - Pro Val Ser Trp Tyr Gly Glu Arg Asp Trp Al - #a Asn Leu

Gly Lys Ser # 130 - Gln His Glu Ala Leu Ala Asp Glu Arg His Ar - #g Leu His Ala Ala
Leu # 145 - His Lys Thr Val Tyr Ile Ala Pro Leu Gly Ly - #s His Lys Leu Pro Glu 150 1
- #55 1 - #60 1 - #65 - Gly Gly Glu Val Arg Arg Val Gln Lys Val Gl - #n Thr Glu Gln
Glu Val # 180 - Ala Glu Ala Ala Gly Met Arg Tyr Phe Arg Il - #e Ala Ala Thr Asp His #
195 - Val Trp Pro Thr Pro Glu Asn Ile Asp Arg Ph - #e Leu Ala Phe Tyr Arg # 210 - Thr
Leu Pro Gln Asp Ala Trp Leu His Phe Hi - #s Cys Glu Ala Gly Val # 225 - Gly Arg Thr
Thr Ala Phe Met Val Met Thr As - #p Met Leu Lys Asn Pro 230 2 - #35 2 - #40 2 - #45 -
Ser Val Ser Leu Lys Asp Ile Leu Tyr Arg Gl - #n His Glu Ile Gly Gly # 260 - Phe Tyr
Tyr Gly Glu Phe Pro Ile Lys Thr Ly - #s Asp Lys Asp Ser Trp # 275 - Lys Thr Lys Tyr
Tyr Arg Glu Lys Ile Val Me - #t Ile Glu Gln Phe Tyr # 290 - Arg Tyr Val Gln Glu Asn
Arg Ala Asp Gly Ty - #r Gln Thr Pro Trp Ser # 305 - Val Trp Leu Lys Ser His Pro Ala
Lys Ala 310 3 - #15 - (2) INFORMATION FOR SEQ ID NO:3: - (i) SEQUENCE CHARACTERISTICS:
#pairs (A) LENGTH: 20 base (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D)
TOPOLOGY: linear - (ii) MOLECULE TYPE: other nucleic acid #= "oligonucleotide SrPr6"
/desc - (iii) HYPOTHETICAL: NO - (iv) ANTI-SENSE: NO - (vi) ORIGINAL SOURCE:
#ruminantium) ORGANISM: Selenomonas (B) STRAIN: JY35 - (vii) IMMEDIATE SOURCE: (A)
LIBRARY: Genomic DN - #A library (B) CLONE: pSrP.2 - (xi) SEQUENCE DESCRIPTION: SEQ ID
NO:3: # 20 GTAT - (2) INFORMATION FOR SEQ ID NO:4: - (i) SEQUENCE CHARACTERISTICS:
#pairs (A) LENGTH: 20 base (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D)
TOPOLOGY: linear - (ii) MOLECULE TYPE: other nucleic acid #= "oligonucleotide SrPf6"
/desc - (iii) HYPOTHETICAL: NO - (iv) ANTI-SENSE: NO - (vi) ORIGINAL SOURCE:
#ruminantium) ORGANISM: Selenomonas (B) STRAIN: JY35 - (vii) IMMEDIATE SOURCE: (A)
LIBRARY: Genomic DN - #A library (B) CLONE: pSrP.2 - (xi) SEQUENCE DESCRIPTION: SEQ ID
NO:4: # 20 CTAC - (2) INFORMATION FOR SEQ ID NO:5: - (i) SEQUENCE CHARACTERISTICS:
#pairs (A) LENGTH: 31 base (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D)
TOPOLOGY: linear - (ii) MOLECULE TYPE: other nucleic acid #= "oligonucleotide MATE2"
/desc - (iii) HYPOTHETICAL: NO - (iv) ANTI-SENSE: NO - (vi) ORIGINAL SOURCE:
#ruminantium) ORGANISM: Selenomonas

Other Reference Publication (10):
Howson, S.J. and Davs, R.P. 1983. Production of Phytase-hydrolysing Enzyme by Some Fungi. Enzyme Microb. Technol. 5:377-382.

Other Reference Publication (18):
Shieh, T.R. and Ware, J.H. 1968. Survey of Microorganisms for the Production of Extracellular Phytase, Appl. Microbiol. 16:1348-1351.

Other Reference Publication (19):
van Hartingsveldt, Wim, van Zeijl, C.M.J. Harteveld, G.M., Gouka, R.J., Suykerbuyk, M.E.G. Luiten, R.G.M., van Paridon, P.A., Selten, G.C.M., Veenstra, A.E., van Gorcum, R.F.M. and van den Hondel, C.A.M.J. 1993. Cloning, Characterization and Overexpression of the Phytase-Encoding Gene (phyA) of Aspergillus niger. Gene 127:87-94.

CLAIMS:

1. A purified and isolated phytase of a ruminal microorganism.

2. A purified and isolated phytase according to claim 1 wherein said ruminal microorganism is Selenomonas ruminantium.

3. A purified and isolated phytase according to claim 2 wherein said phytase has the following characteristics:

a) a molecular mass of about 37 kDa;

b) is active within a pH range of about 3.0 to 6.0; and

c) is active within a temperature range of about 20 to 55.degree. C.

4. A purified and isolated phytase according to claim 3 having the following additional characteristic:

d) a specific activity at least two fold higher than that of Aspergillus ficuum NRRL, 3135 PhyA as measured by the release of inorganic phosphate when assayed by incubating one volume of a sample of the phytase in four volumes of a substrate solution

comprising 0.2% (w/v) sodium phytate in 0.1M sodium acetate buffer (pH 5.0) for 30 minutes at 37.degree. C.

5. A purified and isolated phytase according to claim 1, comprising a contiguous amino acid sequence residing within amino acid sequence SEQ ID NO. 2.

6. A purified and isolated phytase according to claim 1 comprising amino acid sequence SEQ ID NO. 2.

7. A purified and isolated phytase according to claim 1 wherein said ruminal microorganism is a prokaryote.

8. A purified and isolated phytase according to claim 1 wherein said ruminal microorganism is of the genus Selenomonas, Prevotella, Treponema, or Megasphaera.

9. A purified and isolated phytase according to claim 1 wherein said ruminal microorganism is Selenomonas ruminantium, Prevotella ruminicola, Treponema bryantii or Megasphaera elsdenii.

10. A purified and isolated phytase according to claim 1 wherein said ruminal microorganism is Selenomonas ruminantiuni JY35 (ATCC 55785).

11. A purified and isolated phytase according to claim 1, said phytase being encoded by a DNA capable of hybridizing under stringent conditions with a probe comprising at least 25 continuous nucleotides of nucleotide sequence SEQ ID NO. 1 or a complement thereof.

12. A purified and isolated phytase according to claim 1 comprising the amino acid sequence of SEQ ID NO. 2 from amino acid number 10 to amino acid number 319.

13. A purified and isolated phytase according to claim 1 comprising the amino acid sequence of SEQ ID NO. 2 from amino acid number 31 to amino acid number 319.

14. A feed additive for treatment of a feedstuff, said feed additive comprising a purified and isolated phytase according to any one of claims 1-6, or 7-13.

15. A method for improving dietary phytate utilization by an animal, comprising feeding said animal a diet which includes an effective amount of a purified and isolated phytase according to any one of claims 1-6, or 7-13.

16. A feed composition containing a purified and isolated phytase of a ruminal microorganism.

18. A feed composition according to claim 16 wherein said phytase comprises amino acid sequence SEQ ID NO. 2.

19. A feed composition according to claim 16 containing a sufficient amount of the phytase to provide up to about 2000 Units (.mu.moles phosphate released/minute) of phytase activity per kg feed composition.

20. A feed composition according to claim 16 containing a sufficient amount of the phytase to provide up to about 1000 Units of phytase activity per kg feed composition.

21. A feed composition according to claim 16 containing a sufficient amount of the phytase to provide from about 50 to 800 Units of phytase activity per kg feed composition.

22. A feed composition according to claim 16 containing a sufficient amount of the phytase to provide from about 300 to 800 Units of phytase activity per kg feed composition.